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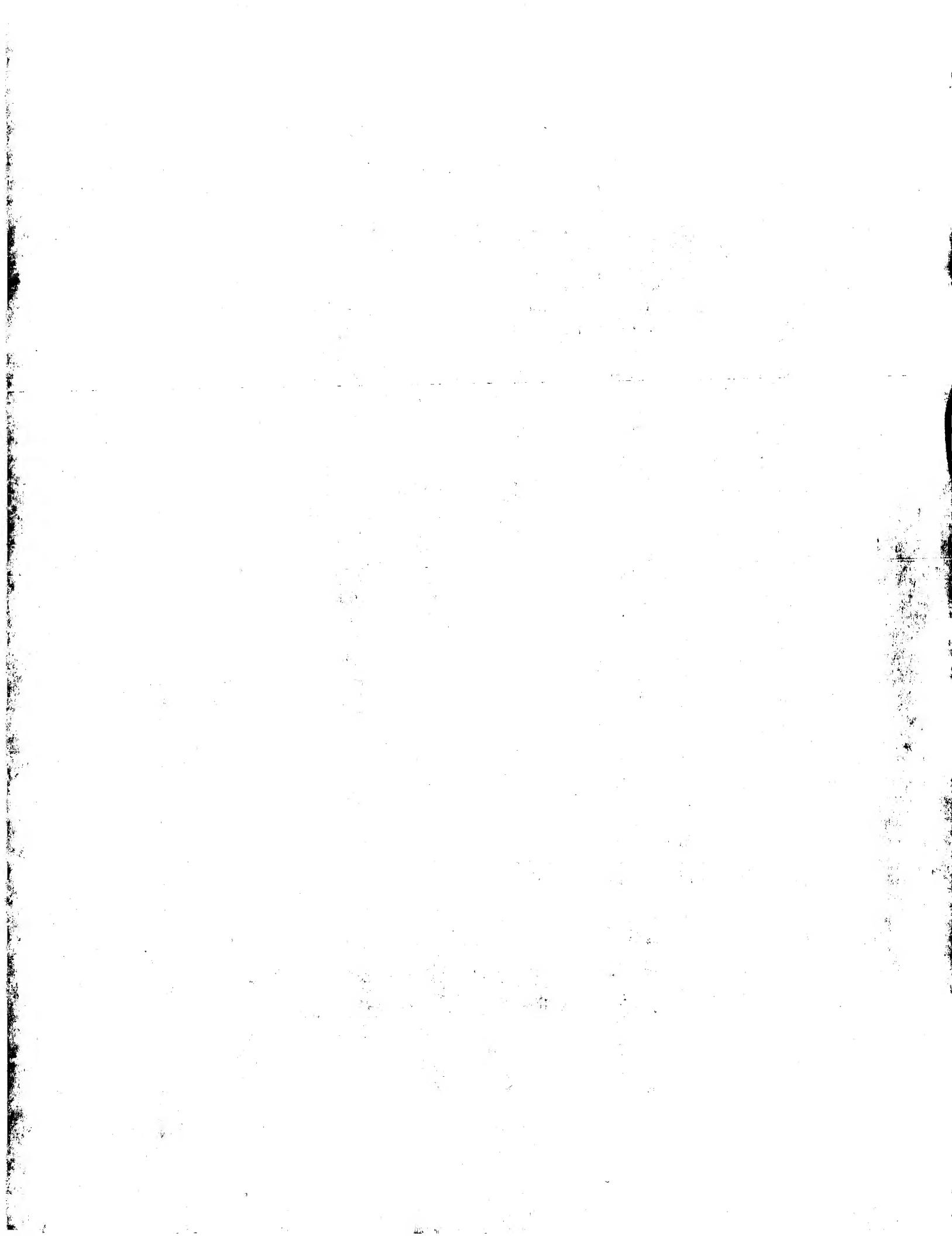
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(57) Abstract

An implant is provided for transplantation to the subretinal area of a host eye comprising a laminate of a monolayer of retinal pigment epithelium (RPE) cells and a non-toxic, flexible support that, upon exposure to a set of predetermined conditions, will not impede normal eye tissue function. A method for preparing a population of RPE cells for transplantation to the subretinal area of a host eye is also provided. The method includes the steps of providing donor tissue comprising RPE cells, harvesting from that tissue RPE cells, and apposing the harvested RPE cells as a monolayer to a non-toxic, flexible support that, upon transplantation to the subretinal area and exposure to a set of predetermined conditions, will not impede normal eye tissue function of the host eye and the transplanted population. A method for transplanting the above implant is also provided, comprising providing the implant, making an incision through a host's eye, at least partially detaching the retina to permit access to the subretinal area, and positioning the implant in the accessed area.

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RETINAL PIGMENT EPITHELIUM TRANSPLANTATION

BACKGROUND OF THE INVENTION

This application is a continuation-in-part of copending application Serial No. 07/566,996 filed August 13, 1990, which in turn is a continuation-in-part of application Serial No. 07/394,377, filed August 14, 1989, now abandoned.

The present invention relates in general to cell and tissue transplantation techniques. More particularly, the present invention is directed to techniques for transplanting populations of retinal pigment epithelium (RPE) cells as a monolayer to the subretinal region of the eye, and to methods for preparing implants comprising monolayers of RPE cells for transplantation.

The retina is the sensory epithelial surface that lines the posterior aspect of the eye, receives the image formed by the lens, transduces this image into neural impulses and conveys this information to the brain by the optic nerve. The retina comprises a number of layers, namely, the ganglion cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, outer nuclear layer, photoreceptor inner segments and outer segments. The outer nuclear layer comprises the cell bodies of the photoreceptor cells with the inner and outer segments being extensions of the cell bodies. The choroid is a vascular membrane containing large branched pigment cells that lies between the retina and the sclerotic coat of the vertebrate eye. Atop the choroid is a membrane 1-5 microns in thickness essentially composed of collagen, known as Bruch's membrane.

Immediately between Bruch's membrane and the retina is the retinal pigment epithelium which forms an intimate structural and functional relationship with the

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photoreceptor cells. Among the functions performed by RPE
cells is the phagocytosis of outer segment debris produced
by the photoreceptors. It is believed that failure of the
RPE cells to properly perform their functions such as

5   digestion of outer segment debris leads to the eventual
degeneration and loss of photoreceptor cells.

In the leading causes of visual impairment in
western industrialized countries, such as age-related
macular degeneration (AMD), both photoreceptors and the

10   underlying RPE are compromised, or have degenerated. A
further aspect of AMD is the frequent appearance of
subretinal neovascular membranes which grow through the
Bruch's membrane and the RPE and tend to hemorrhage and
leak fluids into the subretinal space.

15   In an effort to recover what was previously
thought to be an irreparably injured retina, researchers
have suggested various forms of grafts and transplantation
techniques, none of which constitute an effective manner
for reconstructing a dystrophic retina. The

20   transplantation of retinal cells to the eye can be traced
to a report by Royo et al., Growth 23: 313-336 (1959) in
which embryonic retina was transplanted to the anterior
chamber of the maternal eye. A variety of cells were
reported to survive, including photoreceptors.

25   Subsequently del Cerro was able to repeat and extend these
experiments (del Cerro et al., Invest. Ophthalmol. Vis.
Sci. 26: 1182-1185, 1985). Soon afterward Turner, et al.
Dev. Brain Res. 26:91-104 (1986) showed that neonatal
retinal tissue could be transplanted into retinal wounds.

30   Li and Turner, Exp. Eye Res. 47:911 (1988) have
proposed the transplantation of retinal pigment epithelium
(RPE) into the subretinal space as a therapeutic approach
in the RCS dystrophic rat to replace defective mutant RPE
cells with their healthy wild-type counterparts. According

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to their approach, RPE were isolated from 6- to 8-day old
black eyed rats and grafted into the subretinal space by
using a lesion paradigm which penetrates through the sclera
and choroid. A 1 ml bolus injection of RPE (40,000 -
5 60,000 cells) was made at the incision site into the
subretinal space by means of a 10 ml syringe to which was
attached a 30 gauge needle. However, while this technique
is marginally appropriate for immature RPE cells, with
mature cells it leads to activation and transformation of
10 these cells which damages eye and retinal tissue.

Lopez et al., _Invest. Ophthalmol. Vis. Sci._ 30:
586-589, 1989, also reported a procedure for the
transplantation of dissociated RPE cells. In this
procedure, RPE cells were obtained from normal, congenic,
15 pigmented rat eyes by trypsin digestion. These freshly
harvested, dissociated RPE cells were injected into the
subretinal area of the eyes of dystrophic RCS rats via an
incision through the sclera, choroid and neural retina.
Comparable to the Li and Turner approach discussed above,
20 this procedure destroys the organized native structure of
the transplanted RPE cells, which take the form of a
confluent monolayer in a healthy eye. Moreover, the
procedure is of questionable value for the transplantation
of mature RPE cells. When mature RPE cells are
25 transplanted in dissociated form, experimental results
indicate that they are likely to become activated, migrate
into the subretinal space, and as noted by Lane, C., et
al., _Eye_ (1989) 3, 27-32, invade the retina and vitreous.
This activation of the transplanted, mature RPE cells can
30 result in such pathologies as retinal pucker, massive
subretinal fibrosis, retinal rosette formation, retinal
detachment, and proliferative vitreoretinopathy.

The difficulties discussed above associated with
the transplantation of mature RPE cells is problematic for

human transplantation since available supplies of immature human RPE donor tissue are extremely limited. Moreover, the inability to use mature cells effectively prevents transplantation using autologous RPE tissue, which

5   otherwise would be desirable to avoid the complications involving potential immunological responses faced by non-autologous transplants. Since the victims of AMD are predominantly older adults, in most cases utilizing autologous tissues for transplants would necessarily entail

10   the use of mature human RPE cells.

        It is believed by the present inventor that it is necessary to maintain adult human RPE cells substantially as a monolayer to achieve their successful transplantation and to avoid the problems associated with activation of RPE

15   cells. Although not wishing to be limited to a particular theory, it is thought that the cell-to-cell contact inhibition provided by an intact monolayer, supplemented by adherence to a substrate, mitigates against RPE cell activation. Moreover, a monolayer structure for the RPE

20   provides a proper foundation for the maintenance of the photoreceptor cells in an organized outer nuclear layer structure and for proper growth and arrangement of inner and outer segments, believed by this inventor to be advantagous to restore a reasonable degree of vision. The

25   requirement that the photoreceptors be maintained in an organized structure is based on the well known optical characteristics of photoreceptors (outer segments act as light guides) and clinical evidence showing that folds or similar, even minor, disruptions in the retinal geometry

30   can severely degrade visual acuity.

        Additionally, in cases of AMD where subretinal neovascular membranes have appeared, prior to RPE transplantation, such membranes will need to be removed to prevent subretinal edemas and hemorrhaging of these

membranes.   In practice, removal of the neovascular
membrane results in removal of the native RPE and Bruch's
membrane as well.

　　　A critical impediment to the transplantation of
5   RPE cells as a monolayer is the fragility of the
intercellular structure of RPE relative to the rigors of
manipulation during transplantation to the subretinal
area. Moreover, providing satisfactory support to the RPE
cells during this process is complicated by the fact that
10   the support must either be removed subsequent to
transplantation, to avoid compromising metabolic exchange
between the choroid and the overlying retina, or be
compatible with such ongoing physiological activity. Thus,
a method is needed wherein an implant comprising a
15   monolayer of RPE cells is prepared and transplanted in
which the component supporting the monolayer of RPE cells,
upon transplantation to the subretinal area and exposure to
a set of predetermined conditions, does not impede normal
eye tissue function. Further, a Bruch's-like membrane for
20   attachment of the transplanted RPE cells will be needed in
those cases where neovascularization has occurred and the
native Bruch's membrane is removed.

SUMMARY OF THE INVENTION

　　　Among the objects of the present invention,
25   therefore, may be noted the provision of a method for
preparation of a population of RPE cells as a monolayer for
use in the reconstruction of a dystrophic RPE; the
provision of such a method which allows for use of mature,
and in particular, autologous, mature RPE cells as donor
30   tissue in the reconstruction of a dystrophic RPE; the
provision of an implant for use in the reconstruction of a
dystrophic retina; the provision of such an implant which
does not interfere with normal eye tissue function after

transplantation and maintains photoreceptors and their inner and outer segments by allowing for maintenance of the native organization of the photoreceptor; the provision of such an implant which provides a membrane for attachment of
5   the population of RPE cells especially when the native Bruch's membrane has been removed; and the provision of a method for transplantation of such implants to the subretinal area of an eye.

      Briefly, therefore, the present invention is
10 directed to a method for the preparation of a population of RPE cells for transplantation to the subretinal area of a host eye. The method comprises providing donor tissue comprising RPE cells, harvesting from that tissue RPE cells, and apposing the harvested RPE cells as a monolayer
15 to a non-toxic, flexible support that, upon transplantation to the subretinal area and exposure to a set of predetermined conditions, will not impede normal eye tissue function of the host eye and the transplanted population.

      The present invention is further directed to an
20 implant for transplantation to the subretinal area of a host eye. The implant comprises a laminate of a monolayer of retinal pigment epithelium cells and a non-toxic, flexible support that, upon transplantation to the subretinal area and exposure to a set of predetermined
25 conditions, will not impede normal eye tissue function.

      The present invention is also directed to a method for transplanting to the subretinal area of a host's eye an implant comprising a monolayer of RPE cells. The method comprises providing an implant comprising a laminate
30 of a monolayer of retinal pigment epithelium cells and a non-toxic, flexible support that, upon transplantation to the subretinal area and exposure to a set of predetermined conditions, will not impede normal eye tissue function, making an incision through the host's eye, at least

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partially detaching the retina to permit access to the
subretinal area, and positioning the implant in the
accessed subretinal area.

BRIEF DESCRIPTION OF THE DRAWINGS

5   Fig. 1 is a photograph (200X) of a cryostat
section of a normal rat retina and sub-retinal area;
           Fig. 2(a, b, c, d and e) is a schematic showing
the preparation and transplantation of a preferred
embodiment of an implant comprising a monolayer of RPE
10   cells mounted to a support;
           Fig. 3 is a schematic of an alternative
embodiment of an implant comprising a monolayer of RPE
cells mounted to a support;
           Fig. 4 is a horizontal section through an eye
15   illustrating a transchoroidal and scleral surgical approach;
           Fig. 5 is a horizontal section through an eye
illustrating a transcorneal surgical approach;
           Fig. 6 is a photograph showing a view of the
transplanted implant of an intact monolayer of mature human
20   RPE cells positioned under the retina of a rodent whose
cornea, pupil and lens have been removed, 2 weeks
post-transplantation, as set forth in Example 1;
           Fig. 7 is a photograph (40X) of a section of a
rat retina and sub-retinal area showing an implant of an
25   intact monolayer of a population of mature human RPE cells
14 days after transplantation, as set forth in Example 1;
           Fig. 8 is a higher magnification photograph
(400X) of a section of a rat retina and sub-retinal area
showing an implant of an intact monolayer of a population
30   of mature human RPE cells 14 days after transplantation, as
set forth in Example 1;
           Fig. 9 is a photograph (100X) of a section of a
rat retina and sub-retinal area 14 days after

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transplantation of dissociated mature human RPE cells, as set forth in Example 2; and

Fig. 10 is a higher magnification photograph (300X) of a section of a rat retina and sub-retinal area 14

5   days after transplantation of dissociated mature human RPE cells, as set forth in Example 2.

DETAILED DESCRIPTION

As used herein, the term "donor" shall mean the same or different organism relative to the host and the

10   term "donor tissue" shall mean tissue harvested from or cultured from tissue harvested from the same or different organism relative to the host. Autologous tissue shall mean tissue harvested from or cultured from tissue harvested from the host organism. Mature RPE cells shall

15   mean differentiated RPE cells derived from an organism that is not a fetus.

It is believed that in age-related macular degeneration (AMD), compromise or degeneration of the RPE cells which underlie and form a close structural and

20   metabolic support for the photoreceptors, leads to the loss or destruction of viable photoreceptors. It has been discovered, however, that transplantation of a population of RPE cells as a monolayer, i.e., in essentially a two-dimensional array of cell bodies capable of engaging in

25   cell-to-cell contact inhibition, allows the RPE cells to maintain largely normal characteristics of native, healthy retinal pigment epithelia, capable of providing structural and functional support to photoreceptor cells. Moreover, as illustrated in Example 2 below, it is believed that it

30   is essential to transplant mature RPE cells as a monolayer in order to prevent activation of such cells. Activation leads RPE cells to transdifferentiate into wandering macrophages, fibroblast-like cells and other cell types,

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which cause a number of dystrophic effects in the eye and
retina.

Fig. 1 is a photograph of a cryostat section of a
normal rat retina and subretinal area. In Fig. 1 as well

5   as subsequent figures, the retina or layers thereof, e.g.,
the ganglion cell layer ("G"), inner plexiform layer
("IPL"), inner nuclear layer ("INL"), outer plexiform layer
("OPL"), outer nuclear layer ("ONL"), inner segments
("IS"), outer segments ("OS"), and retinal pigment

10   epithelium ("RPE"), are shown, respectively, from top to
bottom.

Referring now to Figs. 2 and 3, implants
comprising a monolayer of RPE cells are prepared, in
general, by harvesting RPE cells from donor tissue and

15   apposing the harvested RPE cells as an intact monolayer to
a non-toxic, flexible composition, or by seeding such a
composition with a monolayer of dissociated RPE cells and
allowing them to grow into a confluent layer. The flexible
composition serves as a stabilizing support for the RPE

20   cells during transplantation.

The implant constitutes a laminate of a monolayer
of RPE cells and a support composition approximately 100 to
500 microns thick, and preferably 150-250 microns thick.
The surface of the implant has a surface area greater than

25   about 1 square millimeter, preferably greater than 2 square
millimeters, and most preferably greater than 4 square
millimeters, or as large as may be practically handled.
Thus constructed, the implant may subtend a considerable
extent of the sub-retinal surface.

30   In selecting donor tissue for the harvesting of
RPE cells, it is noted that previously, transplantation of
RPE cells was effectively limited to immature cells.
Mature RPE cells transplanted according to prior art
methods have been shown to undergo activation resulting in

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retinal pucker, sub-retinal fibrosis and proliferative
vitreoretinopathy. However, by utilizing the procedures
for implanting a monolayer of RPE cells according to this
invention, transplanted mature RPE cells have successfully

5   maintained essentially normal structure and function.
Thus, the subject invention makes mature RPE cells
available for transplantation, significantly increasing the
supply of usable donor tissue beyond the narrowly limited
supply of immature human donor tissue.

10   It is also noted that the RPE forms part of the
blood-retinal barrier and is thus exposed to lymphocytic
attack. Accordingly, use of autologous RPE cells, now
possible even for mature RPE cells, is preferable, since it
will avoid immunological complications in clinical

15   applications. If non-autologous tissue is utilized, tissue
typing and/or use of an immunosuppression regimen will
generally be necessary to avoid rejection upon
transplantation.

          Donor tissue may be provided, for non-autologous,

20   human RPE tissue, from eye banks, which make the RPE tissue
available in connection with conducting corneal
transplants. Harvesting donated tissue comprising
non-autologous RPE cells can be accomplished by any
suitable method. In general, harvesting of RPE cells may

25   be carried out as set forth in Pfeffer, B.A., Chapter 10,
"Improved Methodology for Cell Culture of Human and Monkey
Retinal Pigment Epithelium", Progress in Retinal Research,
Vol. 10 (1991); Ed. by Osborn, N. and Chader, J., or
Mayerson, P.L., et al., "An Improved Method for Isolation

30   and Culture of Rat Retinal Pigment Epithelial Cells",
Investigative Ophthalmology & Visual Science, Nov., 1985,
26: 1599-1609, which are incorporated herein by reference.
Specifically, to harvest non-autologous RPE cells, a donor
eye is pinned by the optic nerve stump into an eye cage and

placed in an eye jar immediately after corneal removal.
The jar is then flooded to capacity with cold Delbecco's
modified essential medium (DMEM) supplemented with 5% fetal
bovine serum. After loose connective tissue and muscle
5   have been carefully trimmed from the eye, it is placed
upright on a sterile plate and the anterior segment with
the adherent vitreous is lifted out of the eye cup. The
neural retina is separated at the optic disc and removed.
The shell is then washed with Hanks' balanced salt
10   solution, Ca++ and Mg++ free, and treated with 0.25%
trypsin for 30 min. at 37° C. The trypsin solution is
aspirated from the shell and DMEM (GIBCO) is added. The
RPE cells are released from Bruch's membrane by gently
pipetting the culture medium in the shell.
15   For implants containing autologous tissue, RPE
cells may be harvested by performing a biopsy following the
procedures disclosed by Lane, C., et al. in Eye (1989) 3,
27-32.
          The implant also comprises a support for the RPE
20   cells so that the monolayer of RPE cells is less likely to
be damaged and is more easily manipulated during the
transplantation process. The support consists of a
substrate, an overlayer or both, comprising a sheet or
sheets of a non-toxic, flexible composition selected to
25   provide mechanical strength and stability to the easily
damaged monolayer of RPE cells. Because the support
composition will be inserted into the eye as a laminate
with the monolayer of RPE cells, the support is also
comprised so that, upon transplantation to the subretinal
30   area and exposure to a set of predetermined conditions,
described herein, the support composition will not impede
normal eye tissue function of the host eye or the
population of transplanted RPE cells. If the harvested RPE
cells are to be cultured before transplantation, the

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composition providing support to the monolayer of RPE cells during transplantation may optionally be capable of serving as the attachment substrate for the RPE cells during culturing.

5   A variety of compositions may be used as the support for the population of RPE cells, depending upon the specific set of conditions to which the composition will be exposed after transplantation. In short, the composition is selected either because any portion remaining within the

10   subretinal area for more than approximately one week after transplantation is compatible with normal eye tissue function upon exposure to bodily fluids within the sub-retinal area, or for its susceptibility to elimination upon exposure to prescribed conditions.

15   An additional factor to consider in determining the make-up of compositions for support of the monolayer of RPE cells is whether Bruch's membrane has been or will be removed from the host eye prior to transplantation of the monolayer of RPE cells. Bruch's membrane serves to anchor

20   the RPE cells in a healthy eye. Removal of Bruch's membrane may occur in cases of AMD where a subretinal neovascular membrane has formed and Bruch's membrane is removed as a consequence of the removal of the neovascular membrane.

25   In cases where Bruch's membrane is removed, the support composition will comprise a layer of collagen less than 100 microns in thickness, and preferably between 1 and 10 microns in thickness. The basal surfaces of the RPE cells attach readily to the collagen layer, which serves to

30   anchor the RPE cells to the choroid in place of the removed Bruch's membrane. The layer of collagen also serves to inhibit the occurrence or reoccurrence of subretinal neovascularization through and around the transplanted RPE. Such a collagen layer is retained indefinitely in the

sub-retinal area. However, it is known that Bruch's membrane is essentially comprised of collagen, and such microthin layers of collagen are permeable enough to avoid impeding normal eye tissue functions such as the metabolic

5 exchange between the choroid and the retina. However, collagen compositions of such a minimal thickness are not strong enough to prevent buckling or distortion of the monolayer of RPE cells during transplantation. Thus, such microthin collagen materials need to be used in combination

10 with additional supporting materials which will be eliminated after transplantation, if they are to form part of the support for the transplanted RPE cells.

If transplantation is carried out with the native Bruch's membrane intact, the host's RPE cells covering the

15 area to receive the transplant of RPE cells may be physically and/or chemically debrided from the Bruch's membrane, for example, by applying collagenase to the native Bruch's membrane to loosen the host's RPE cells in order to permit their removal. The transplanted RPE cells

20 may then anchor themselves directly to the Bruch's membrane.

Either to supplement a layer of collagen, as discussed above, or in cases where inclusion of a collagen anchor is not required, a support composition may be selected which is dissipated, for example, by exposure to a

25 sufficient amount of heat, selected enzymes, or bodily fluids. Gelatin is an example of a preferred support material which is flexible, lacks toxicity to neural tissue and has the ability to dissolve at body temperature. The support composition may also comprise a material such as

30 activated low gelling temperature agarose (Type VII, #A 4018 Sigma Chemical Co., St. Louis) or fibrin, which are known to dissipate when exposed to selective enzymes which will not attack other compositions. For example, agarase is an enzyme known to specifically attack agarose, and

urokinase is an enzyme specific to fibrin. Another alternative is to use biodegradable polymers such as ethylene-vinyl acetate copolymer (Elvax 40W, DuPont Chemical Co., Delaware); poly(glycolic) acid,

5   poly(L-lactide-co-glycolide)(70:30 ratio) or poly(L- or DL-lactic) acids (low mol. wt.) (Polysciences, Inc., Warrington, PA., Data Sheet #365, 1990), which are flexible, non-toxic materials that slowly dissipate upon implantation and exposure to bodily fluids. See, e.g.,

10 Powell, E.M., Brain Research, 515 (1990) 309-311 and references cited in Polysciences, Inc. Data Sheet #365.

Advantageously, the gelatin or other support composition may additionally serve as a carrier for any of a number of trophic factors such as pharmacologic agents

15 including immunosuppressants such as cyclosporin A, anti-inflammation agents such as dexamethasone, anti-angiogenic factors, anti-glial agents and anti-mitotic factors. Upon dissolution of the support composition, the factor or agent becomes available to impart the desired

20 effect upon the surrounding tissue. The dosage can be determined by established experimental techniques.

With appropriate enzymatic digestion, using techniques disclosed by Pfeffer, B.A., Chapter 10, "Improved Methodology for Cell Culture of Human and Monkey

25 Retinal Pigment Epithelium", Progress in Retinal Research, Vol. 10 (1991), at p.264, RPE cells are removed from the Bruch's membrane as intact sheets rather than as dissociated cells. Such a freshly harvested intact monolayer of RPE cells may be immediately apposed as an

30 intact monolayer to a support, such as a thin collagen sheet, supplemented with an overlayer or substrate of gelatin, allowing several hours for adhesion prior to transplantation. This procedure may be useful for transplantation of RPE cells obtained in a biopsy, provided

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sufficient RPE tissue is obtained from the biopsy as an intact monolayer.

In most cases, however, it is preferable to culture the harvested RPE cells on an attachment substrate
5   so that a monolayer of RPE cells may be prepared. Culturing the RPE cells before harvesting is also preferred both to allow for the production of larger populations of RPE cells from a small amount of harvested tissue and to allow for a period of observation to ensure that the RPE
10   cells to be transplanted are healthy and functional.

For proper culturing, harvested RPE cells contained are apposed to a substrate to which they will attach and grow, and which is capable of being maintained in culture conditions appropriate for efficient growth of
15   RPE cells. Apposition of RPE cells may be accomplished by pipetting a solution containing a population of RPE cells onto the substrate. Intact sheets of harvested RPE cells in a physiological solution may also be physically released via a wide-bore pipette onto the substrate and manipulated
20   with a fine camel-hair brush if necessary. Since RPE cell cultures grow best at around 37° C, appropriate substrates are solids at this temperature. Attachment substrates which are suitable for growth of RPE cell cultures include plastic culture ware or glass culture chamber slides coated
25   with collagen , laminin or fibronectin for cell attachment. Coatings such as collagen may be applied to a substratum such as fibrin or activated agarose.

Culturing harvested RPE cells can be accomplished by any suitable method for obtaining a confluent monolayer
30   of RPE cells. Appropriate culturing techniques are described in Pfeffer, B.A., Chapter 10, "Improved Methodology for Cell Culture of Human and Monkey Retinal Pigment Epithelium", Progress in Retinal Research, Vol. 10 (1991); Ed. by Osborn, N. and Chader, J., or Mayerson,

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P.L., et al., "An Improved Method for Isolation and Culture
of Rat Retinal Pigment Epithelial Cells", _Investigative
Ophthalmology & Visual Science_, Nov., 1985, 26: 1599-1609.

5   Fig. 2 depicts a schematic of the preparation and
transplantation of a preferred embodiment of the implant
according to the invention. An implant laminate 1 is
formed as depicted in Figs. 2a and 2b. Gelatin 3 is
preferred for use to provide monolayer support during
transplantation because it is flexible, non-toxic to neural
10   tissues, and because it dissolves away after exposure to
body temperature (37° C). However, since RPE grows too
slowly at temperatures below 37° C, gelatin is not an
appropriate substrate for use in culturing RPE cells.
Thus, a monolayer of RPE cells 5 is first cultured on a
15   suitable substrate for growth. RPE cells will grow on
plastic culture ware A. However, to aid in removal of the
monolayer of RPE cells 5 from the culture ware A, the
culture ware A may be coated with a substrate 9 for the RPE
cells, such as agarose or fibrin. See Fig. 2b. The
20   substrate 9 is in turn coated with a thin (@ 2-4 microns)
layer of collagen 7, to which the monolayer of RPE cells 5
readily attach, and which will serve as an anchor for the
RPE cells in the post-transplantation period. If agarose
is used as the substrate 9, the agarose is activated so
25   that the collagen 7 will adhere to it by treating the
surface of the agarose with an activator such as cyanogen
bromide, as disclosed by Axen, R. et al., _Nature_ (1967)
214: 1302-1304, or p-nitrophenyl chloroformate as disclosed
by Wilchek, M. and Miron, T., _Biochemistry International_
30   (1982) 4: 629-635.

After a satisfactory culture of RPE cells has
been grown, a 5 to 30% solution of molten gelatin is
applied to the apical surface of the monolayer of RPE cells
5 in the culture. The culture ware containing the RPE

cells and gelatin solution is then cooled so that the
gelatin 3 is solidified into a sheet of preferably about
150-250 mm in thickness, attached to the apical surface of
the RPE cells 5.   The implant laminate 1 comprising a

5   monolayer of RPE cells 5, attached to the collagen anchor 7
and an overlayer of gelatin 3, may then be physically
removed from the culture ware A by slicing between the
culture ware A surface and the collagen 7 with a razor C,
as depicted in Fig. 2a.

10   The implant laminate 1 may also be removed from
the culture ware and substrate 9 enzymatically, as depicted
in Fig. 2b.   The underlying substrate 9 may be removed from
the implant laminate 1 by application of an enzyme specific
to the material comprising the substrate 9.   If the

15   substrate is agarose, agarase, an enzyme specific to
agarose, may be used to dissipate the agarose.   A
concentration of 1 mg agarase/5 ml of culture medium will
suffice to dissipate the agarose substrate.   If the
substrate consists of a fibrin coating, enzymes such as

20   urokinase (2 activity units urokinase/ml of culture media)
may be used to break apart the fibrin.   To aid in
dissipation of the substrate 9, the culture ware A may
comprise a cell culture insert membrane, containing an
inner membrane A-1 with a porous bottom, and an outer,

25   solid membrane A-2.   An example of such an insert membrane
is the Falcon® Cyclopore™ membrane.   The porous (e.g., pore
size of .45 mm) inner membrane A-1 is coated with the
substrate 9 during growth of the monolayer of RPE cells 5.
These pores facilitate penetration of the solution B

30   containing the enzyme into the substrate 9 so that it may
be broken apart, and the implant laminate 1 removed from
the culture ware A.

         As depicted in Fig. 2c, the implant laminate 1 is
cut to create an implant 1a sized so that it will fit into

a surgical instrument 20 which contains a carrier channel
for protection of the implant 1a during the transplantation
procedure.   The implant 1a is transplanted to the
subretinal area at the posterior pole 76 of the host eye

5   after detachment of the retina, as portrayed in Fig. 2d,
using surgical techniques more fully disclosed below. The
gelatin overlayer dissolves within hours after insertion of
the implant 1a into the host eye.   After transplantation,
the retina reattaches, and the monolayer of RPE cells,

10 anchored to the layer of collagen, is sandwiched between
the choroid and the retina. See Fig. 2e.

Fig. 3 depicts a schematic of an alternative
embodiment of the implant according to the invention. As
described in relationship to Fig. 2 above, a monolayer of

15 RPE cells 5 is grown on culture ware coated with a
substrate 9. However, in this embodiment, the substrate 9
remains laminated to and comprises part of the implant 1a
during transplantation.   The substrate 9 used for
transplantation may comprise agarose, which is biologically

20 inert and as discussed previously, may be dissipated by
exposure to agarase, an enzyme which selectively attacks
agarose. In this embodiment, activated agarose is coated
with a thin layer of collagen 7, about 2-4 microns in
thickness, which binds strongly to the activated agarose.

25 A monolayer of RPE cells 5 is then apposed to the collagen
7 and attaches readily to form a laminate of the RPE cells
5 and the support composition (collagen 7 and substrate 9)
to be used as an implant 1a for transplantation. Rather
than adding an overlayer of gelatin as discussed in

30 reference to Fig. 2, a razor is used to slice a sheet of
the material comprising the substrate 9 about 150-250 mm
thick to serve as a stabilizing support for the monolayer
of RPE cells 5 and the collagen 7 during transplantation.

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Once transplantation is completed and the
monolayer of RPE cells has been stabilized in its proper
position, an intraocular injection of an enzyme such as
agarase (1 mg/5 ml vol. of vitreal chamber) if an agarose
5   substrate is used may be administered to break down the
substrate. Alternatively, the enzyme may be incorporated
into a slow-release agent 11 such as ethylene-vinyl acetate
copolymer (Elvax 40W, DuPont Chemical Co., Del.), which is
added to the substrate 9 prior to transplantation. Methods
10   for using biodegradable polymers as slow-release matrices
for enzymes are known in the art, such as disclosed by
Powell, E.M., Brain Research (1990) 515:309-311; Wise, et
al.; Chapter 12, "Lactic/Glycolic Acid Polymers," Drug
Carriers in Biology and Medicine (Ed., Gregoriades), 1979;
15   Kitchell, J. and Wise, D., "Poly(lactic/glycolic acid)
Biodegradable Drug - Polymer Matrix Systems," Methods in
Enzymology, 112:436-448 (1985). Slow release of the enzyme
causes gradual break up of the substrate support. The
collagen 7 remaining after dissipation of the substrate 9
20   is physiologically permeable to and does not impede normal
tissue function, and serves to anchor the monolayer of RPE
cells 5, as well as to inhibit neovascularization in the
subretinal area of the host eye.

Fibrin, discussed in connection with Fig. 2, may
25   also be used as the substrate 9 in the embodiment depicted
in Fig. 3. Fibrin is the fibrous, insoluble protein that
forms the structural component for blood clots. Fibrin may
be pressed to add tensile strength and is a material which
provides a flexible, non-toxic support to which RPE cells
30   will attach and grow as a monolayer. To prepare fibrin,
fibrin clot suitable for substrate use is made by
catalyzing the polymerization of fibrinogen into fibrin by
the addition of thrombin. After transplantation, fibrin
will dissipate upon exposure to naturally produced enzymes

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which dissolve blood clots. If the dissipation of the
fibrin support composition is to be accelerated, enzymes
which are specific for fibrin, such as tissue plasminogen
activator (TPA), urokinase or streptokinase may be
5   administered using one of the methods for application of
enzymes discussed above.

        To transplant the implant, the host eye is
prepared so as to reduce bleeding and surgical trauma. A
transcleral/transchoroidal surgical approach to the
10   subretinal space is an example of a suitable approach and
it will be understood that other surgical approaches, such
as a transcorneal approach, may also be used. The
preferred surgical approach in the human, Fig. 4, includes
making a transverse incision 70 in a sclera and choroid 78
15   of sufficient size so as to allow insertion of a surgical
instrument 20. The instrument 20 is advanced through the
sclera and choroid 78 and to the ora serrata 74 as
illustrated in Fig. 4. The instrument detaches the retina
as it is advanced under the retina and into the sub-retinal
20   space to the posterior pole 76 of the eye.

        Preferably, an instrument comprising an elongate
tube having a flat, wide cross-section may be used so that
the implant may be drawn into the elongate tube for
protection as it is transported through an appropriate
25   sized incision in the sclera or choroid. The instrument is
advanced to the ora serrata 74 of the host eye and if the
instrument includes a lumen, the retina is detached by the
gentle force of a perfusate such as a saline-like fluid,
carboxymethylcellulose, or 1-2% hyluronic acid ejected from
30   the lumen. Advantageously, the fluid may additionally
contain anti-oxidants, anti-inflammation agents,
anesthetics or agents that slow the metabolic demand of the
host retina.

        If the instrument does not include a lumen, the
35   retina is detached by subretinal irrigation or by the walls

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of the surgical instrument as it is advanced under the
retina and into the subretinal space to the posterior pole
76 of the eye. The implant is then transplanted by
retracting the tube containing the implant from the eye
5   while simultaneously gently ejecting the implant from the
tube. The instrument is then carefully withdrawn out of
the eye. Retinal reattachment occurs rapidly and the
monolayer of RPE cells is held in place in a sandwich-like
arrangement between the retina and the choroid. The
10   incision may require suturing.

Fig. 5 depicts a transcorneal surgical approach
as an alternative to the transscleral and choroidal
approaches described above. Except for the point of entry,
the surgical technique is essentially the same as outlined
15   for the transscleral or choroidal approaches. A transverse
incision 70 is made in a cornea 72 and the instrument 20
containing the implant is advanced under the iris, through
the cornea 72 and to the ora serrata 74 as illustrated in
Fig. 5. The iris should be dilated for example, with
20   topical atropine. The edges of the corneal incision are
abutted and sutured if necessary to allow healing. The
transcorneal approach is preferred for rodents because it
has been found to reduce bleeding and surgical trauma.
Nevertheless, a transscleral or choroidal approach is
25   preferred for humans to avoid scarring of the cornea which
may interfere with visual acuity.

A further surgical approach is to diathermize in
the pars planna region to eliminate bleeding. The sclera
is then incised and the choroidal and any native epithelial
30   tissue is diathermized. The surgical tool is then inserted
through the incision, the retina is intercepted at the ora
serrata and the implant is deposited in the subretinal area
otherwise as outlined elsewhere herein.

In yet a further surgical approach, entry is
35   gained through the pars planna area as outlined above and

an incision is made in the retina adjacent to the retinal
macula. The surgical tool is then inserted through the
retinotomy and into the macular area.

5   As discussed previously in connection with the
make up of the support compositions used in accordance with
the invention, after transplantation, the implant is
exposed to a predetermined set of conditions, such as
exposure to heat, selected enzymes or bodily fluids. Upon
the appropriate amount of exposure, the support composition
10   is dissipated or will not otherwise impede normal eye
tissue function of the host eye and the transplanted
population of RPE cells.

       The following examples illustrates the invention.

                           EXAMPLE 1

15   Experimental Animal and Materials

       RPE cells were taken from the sub-retinal area of
donated human eyes (obtained from the Missouri Lions and
St. Louis Eye Banks) following corneal removal. Hosts were
adult albino rats immune-suppressed with cyclosporin A 10
20   mg/kg/day IP injection).

       Harvesting of RPE Cells

       Immediately after corneal removal, the eye was
pinned by the optic nerve stump into an eye cage and placed
into an eye jar. The jar was flooded to capacity with cold
25   Delbecco's modified essential medium (DMEM), supplemented
with 5% fetal bovine serum. The eye was processed in a
sterile environment. After loose connective tissue and
muscle were carefully trimmed from the eye, it was placed
upright on a sterile plate and the anterior segment with
30   the adherent vitreous was lifted out of the eye cup. Four
slits were cut radially toward the optic disc with the eye
lying flat in a petri dish. The neural retina was then

separated at the optic disc and removed. The shell was
then washed with Hanks' balanced salt solution, Ca++ and
Mg++ free, and treated with 0.25% trypsin for 30 min. at
37° C. The trypsin solution was aspirated from the shell
5 and DMEM (GIBCO) was added. The cells were released from
Bruch's membrane by gently brushing the surface of the RPE
cells with the polished tip of a pasteur pipette and
pipetting the culture medium in the shell.

Culturing of RPE Cells

10   Glass culture chamber slides were prepared by
coating them with liquid collagen obtained from Sigma
Chemical Co., St. Louis. The collagen was allowed to
solidify to about 150 microns in thickness. 3×10^5 RPE
cells were plated onto the prepared slides using a pasteur
15 pipette. The RPE cells were then incubated in DMEM + F12
(1:1. GIBCO) supplemented with 20% fetal bovine serum
(Sigma Chemical Co., St. Louis). The culture medium was
changed every 3-4 days until a confluent monolayer of RPE
cells had been cultivated. Implants comprising monolayers
20 of RPE cells laminated to collagen were removed from the
culture chamber slides and sheets of approximately 2 mm X 4
mm were cut out for transplantation.

Surgical Procedure

           A transverse incision was made in the cornea
25 sufficient to allow insertion of a surgical instrument that
is 2.5 mm wide with a lumen 0.5 mm high. The instrument
was advanced under the iris (dilated with topical atropine)
to the ora serrata, detaching the retina. The carrier was
then advanced under the retina into the subretinal space to
30 the posterior pole of the eye. The instrument allowed an
implant comprising a monolayer of RPE cells apposed to a
150 mm collagen substrate (up to 2.5 X 4 mm) to be guided

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into the retinal space by advancement of the plunger with
simultaneous retraction of the surgical instrument. The
instrument was then removed. Following removal of the
instrument, the edges of the corneal incision were abutted
5   to allow rapid, sutureless healing. The eye was patched
during recovery and a prophylactic dose of penicillin was
administered. Upon removal of the patch, a veterinary
ophthalmic antibiotic ointment was applied.

          Transplant recipients were maintained on a 12
10   hr/12 hr light/dark cycle with an average light intensity
of 50 lux. Following appropriate survival times, the
animal was overdosed with pentobarbital.

          Fig. 6 shows a view of the transplanted monolayer
of RPE cells positioned under the retina, with the cornea,
15   pupil and lens removed, two weeks post-transplantation.
The arrows designate the edges of the implant.

Histologic Preparation

          For histologic evaluation, rat eyes with RPE cell
implants were immersion-fixed in Bouin's solution for 5
20   hrs., then dehydrated in a graded ethanol series. The eyes
were trimmed with a razor blade to include the transplanted
area for implanted retinas, then processed through xylene
and embedded in paraffin. Sections were cut at 8mm and
stained with hematoxylin and eosin.

25   Results

          By using the transcorneal approach, it was found
that the positioning of the monolayer of RPE cells between
the host's retina and the adjacent choroidal tissue layer
of the eye could be accomplished while minimizing the
30   vascular damage and subsequent bleeding into the eye. In
addition, it was found that this approach does not appear
to disrupt the integrity of the retina, which reattaches to

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the back of the eye with the transplanted RPE cells
interposed between the retina and the choroid. Using this
insertion method, it was possible to position the RPE cells
beneath the posterior pole of the retina (Fig. 7).

5   To determine the viability of the transplanted
RPE cells, paraffin sections (8 mm) were made from the eye
receiving the RPE cell transplant at 2 weeks, 4 weeks, and
3 months after transplantation. It was found that the
monolayer of RPE cells survived transplantation at all
10   times tested (10 out of 12 transplants).

               With immune-suppression, successful transplants
were seen at all survival times so far examined (2 weeks to
3 months), showing apparent physical integration with the
host eye and maintaining morphological features of the RPE
15   as illustrated in Fig. 7 which shows a transplant of a
monolayer of human RPE cells from adult donor to adult rat
host. (T, transplant). 40X.

               Paraffin sections made at 2 weeks post-
transplantation are shown in Figs. 7 and 8. Fig. 7 is a
20   low-power photomicrograph showing the location of the RPE
monolayer transplant (T) between arrowheads at the
posterior pole of th host eye. Note the maintenance of
normal retinal configuration as well as the normal
appearance of the RPE cells, maintained in substantially a
25   monolayer between the choroid and the outer segments of the
photoreceptor cells. Figure 8 is a higher-power
photomicrograph showing in detail the interface between the
transplant and the adjacent retina. As shown in Fig. 8,
the photoreceptor outer segments are shown to be in normal
30   apposition to the apical microvillar processes (A) of
transplanted RPE cells.

               The functional capabilities of the transplanted
RPE cells and reconstructed retina were ascertained by
their maintenance of photoreceptor bodies, and the presence

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of inner and outer segments with proper orientation to the apical processes of transplanted RPE cells. The success of these procedures for transplanting monolayers of RPE cells are reflected in Figs. 7 and 8.

5   EXAMPLE 2

As a comparison, mature human RPE cells were transplanted in dissociated form using the bolus injection method as set forth by Li and Turner in Exp. Eye Res., 47:911 (1988). RPE cells from the same donor tissue used
10   for the transplants conducted as set forth in Example 1 were harvested and cultured as set forth in Example 1. After culturing, the RPE cells were trypsonized to form dissociated RPE cells for transplantation by bolus injection according to the method of Li and Turner. Hosts
15   were adult albino rats immune-suppressed with cyclosporin A 10 mg/kg/day IP injection) as set forth in Example 1.

Transplant recipients were maintained on a 12 hr/12 hr light/dark cycle with an average light intensity of 50 lux. Following appropriate survival times, the
20   animal was overdosed with pentobarbital. Paraffin sections of the eye receiving the RPE cell implant were then cut (8 mm).

Paraffin sections made at 2 weeks post-transplantation are shown in Figs. 9 and 10. Fig. 9 is a
25   low-power photomicrograph showing the location of the injection of dissociated RPE cells at the posterior pole of the host eye. Note the pathological configurations, including retinal detachment (RD), retinal pucker (RP), subretinal fibrosis (SF) and retinal rosette (RR)
30   formation. Figure 10 is a higher-power photomicrograph showing in detail the pathological invasion of RPE cells into the adjacent retina, subretinal fibrosis and retinal pucker.

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From the foregoing description those skilled in the art will appreciate that all aspects of the present invention are realized. The present invention provides an improved surgical implant that is adapted to provide cell

5   organization during transplantation of the RPE cells. With the implant of this invention cell organization is maintained during and after RPE transplantation.

In view of the above, it will be seen that the several objects of the invention are achieved and other

10   advantages attained.

As various changes could be made in the above compositions and methods without departing from the scope of the invention, it is intended that all matter contained in the above description or shown in the accompanying

15   drawings shall be interpreted as illustrative and not in a limiting sense.

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WHAT IS CLAIMED

1. A method for the preparation of a population
of RPE cells for transplantation to the subretinal area of
an eye of a host comprising:

(a) providing donor tissue comprising RPE cells,

5   (b) harvesting RPE cells from the donor tissue,
and

(c) apposing the harvested RPE cells as a
monolayer to a non-toxic flexible support that, upon
transplantation to the subretinal area and exposure to a
10   set of predetermined conditions, will not impede normal eye
tissue function of the eye of the host and the transplanted
population.

2. A method as set forth in claim 1 further
comprising culturing the harvested RPE cells.

3. A method as set forth in claim 1 wherein the
donor tissue comprises mature RPE cells.

4. A method as set forth in claim 1 wherein the
donor tissue is autologous to the host.

5. A method as set forth in claim 1 wherein the
support comprises a layer of collagen less than about 100
microns in thickness.

6. A method as set forth in claim 1 wherein the
harvested RPE cells have apical and basal surfaces, the
support further comprises a substrate of collagen and an
overlayer of gelatin, and the basal surfaces of the
5   harvested RPE cells are apposed to the collagen and the
apical surfaces of the harvested RPE cells are apposed to
the gelatin.

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7.   A method as set forth in claim 1 wherein the harvested RPE cells have an apical surface and apposition of the RPE cells to the support further comprises contacting the apical surface of the RPE cells to the
5   support.

8.   A method as set forth in claim 1 wherein said support comprises a material degradable by exposure to heat, bodily fluids or enzymatic action.

9.   A method as set forth in claim 1 wherein the support further comprises an enzyme adapted to break down at least a portion of the support after positioning of the implant in the subretinal area.

10.   A method as set forth in claim 9 wherein the enzyme is incorporated into a slow release agent.

11.   A method as set forth in claim 10 wherein the slow release agent is a biodegradable polymer.

12.   A method as set forth in claim 10 wherein the slow release agent is selected from the group consisting of polylactic acid, polyglycolic acid, polylactide-co-glycolide and ethylene-vinyl acetate
5   copolymer.

13.   A method as set forth in claim 1 wherein the support comprises a sheet of agarose coated with a layer of collagen between about 1 and about 100 microns in thickness and wherein the harvested RPE cells are apposed to the
5   collagen prior to transplantation.

14.   A method as set forth in claim 13 wherein the support further comprises agarase incorporated into a slow release agent.

15.   A method as set forth in claim 1 wherein the support comprises fibrin.

16.   A method as set forth in claim 15 wherein the support further comprises an enzyme selected from the group of tissue plasminogen activator, urokinase or streptokinase.

17.   A method as set forth in claim 1 wherein the support comprised one or more of a trophic factor, an immunosuppressant, an anti-inflammation agent, an ant-angiogenic factor, an anti-glial agent or an
5   anti-mitotic factor.

18.   A method for the preparation of a population of RPE cells for transplantation to the subretinal area of an eye of a host comprising:
        (a)   providing donor tissue comprising RPE cells,
5   (b)   harvesting RPE cells from the donor tissue,
        (c)   culturing the harvested RPE cells on a substrate suitable for attachment and growth of a monolayer of RPE cells,
        (d)   removing the monolayer of cultured RPE cells
10 from the substrate, and
        (e)   apposing the monolayer of cultured RPE cells to a non-toxic flexible support that, upon transplantation to the subretinal area and exposure to a set of predetermined conditions, will not impede normal eye tissue
15 function of the eye of the host and the transplanted population.

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19. A method as set forth in claim 18 wherein
the substrate is comprised of agarose, removing the
monolayer from the substrate comprises contacting the
agarose with agarase, the support comprises gelatin and
5   apposing the monolayer to the support comprises contacting
the monolayer with a molten solution of gelatin and cooling
the gelatin so that the gelatin solidifies.

20. An implant for transplantation to the
subretinal area of an eye of a host comprising a laminate
of a monolayer of RPE cells and a non-toxic, flexible
support that, upon transplantation to the subretinal area
5   and exposure to a set of predetermined conditions, will not
impede normal eye tissue function.

21. An implant as set forth in claim 16 wherein
the RPE cells further comprise mature RPE cells.

22. An implant as set forth in claim 16 wherein
the RPE cells are autologous to the host.

23. An implant as set forth in claim 20 wherein
the predetermined conditions comprise exposure to heat,
bodily fluids or enzymatic action to break down at least a
portion of the composition.

24. An implant as set forth in claim 20 wherein
the support comprises a material degradable by exposure to
heat, bodily fluids or enzymatic action.

25. An implant as set forth in claim 20 wherein
the support further comprises an enzyme adapted to break
down at least a portion of the support after positioning of
the implant in the subretinal area.

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26. An implant as set forth in claim 25 wherein the enzyme is incorporated into a slow release agent.

27. An implant as set forth in claim 26 wherein the slow release agent is a biodegradable polymer.

28. An implant as set forth in claim 26 wherein the slow release agent is selected from the group consisting of polylactic acid, polyglycolic acid, polylactide-co-glycolide and ethylene-vinyl acetate
5   copolymer.

29. An implant as set forth in claim 20 wherein the support comprises a layer of collagen less than about 100 microns in thickness.

30. An implant as set forth in claim 20 wherein the monolayer of RPE cells have apical and basal surfaces, the support further comprises a substrate of collagen and an overlayer of gelatin, and the basal surfaces of the
5   monolayer of RPE cells are laminated to the collagen and the apical surfaces of the monolayer of RPE cells are laminated to the gelatin.

31. An implant as set forth in claim 20 wherein the support comprises a sheet of agarose coated with a layer of collagen between about 1 and about 100 microns in thickness.

32. An implant as set forth in claim 31 wherein the support further comprises agarase incorporated into a slow release agent.

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33. An implant as set forth in claim 20 wherein the support comprises fibrin.

34. An implant as set forth in claim 33 wherein the support further comprises an enzyme selected from the group of tissue plasminogen activator, urokinase or streptokinase.

35. An implant as set forth in claim 20 wherein the support comprises one or more of a trophic factor, an immunosuppresant, an anti-inflamation agent, an anti-angiogenic factor, an anti-glial agent or an
5   anti-mitotic factor.

36. A method for transplanting a monolayer of RPE cells to the subretinal area of an eye of a host comprising:
        (a) providing an implant comprising a laminate
5   of a monolayer of RPE cells and a non-toxic, flexible support that, upon transplantation to the subretinal area and exposure to a set of predetermined conditions, will not impede normal eye tissue function,
        (b) making an incision through the eye of the
10 host,
        (c) at least partially detaching the retina to permit access to the subretinal area, and
        (d) positioning the implant in the accessed subretinal area.

37. A method for transplanting as set forth in claim 36 in which the predetermined conditions comprise exposing the support to heat, bodily fluids, or enzymatic action.

38.   A method for transplanting as set forth in claim 36 further comprising infusing the subretinal area of the eye of the host with an enzyme to break down at least a portion of the support.

FIG.1

G
IPL
INL
OPL
ONL
IS
OS
RPE
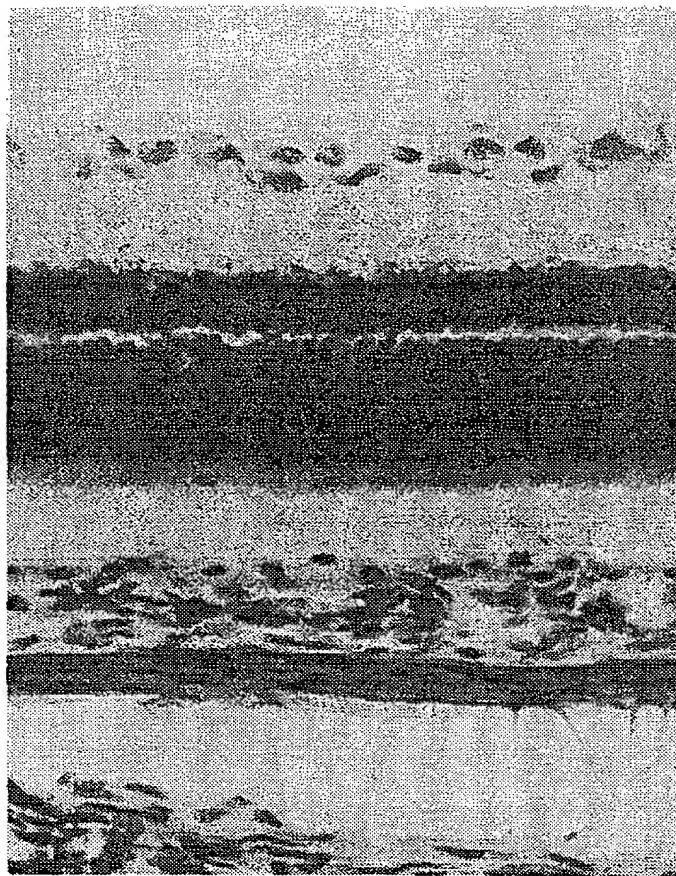
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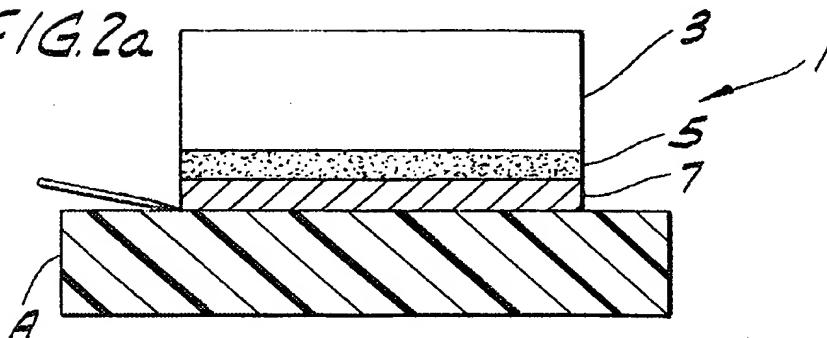
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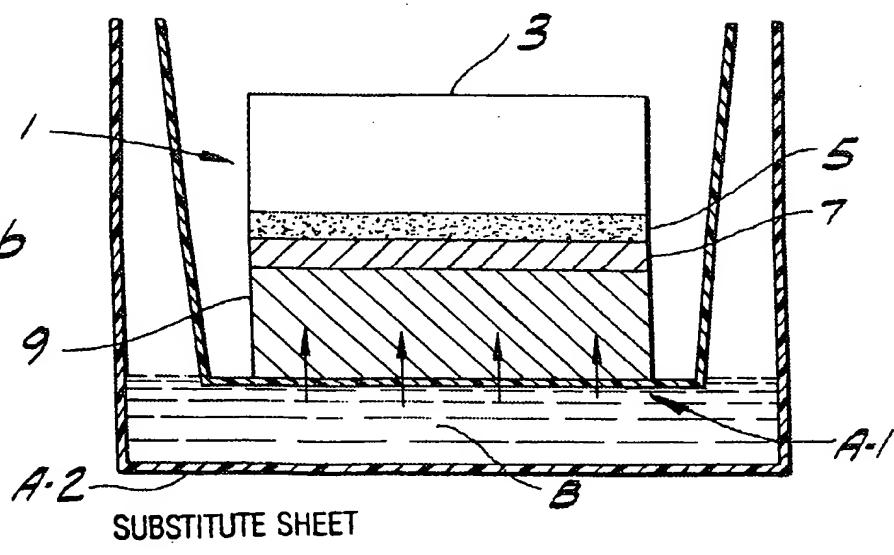
SUBSTITUTE SHEET

FIG.2c
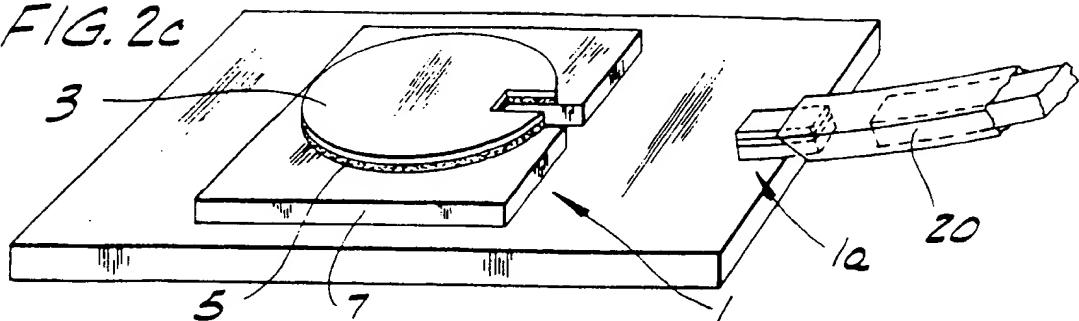
FIG.2d

FIG.2e

SUBSTITUTE SHEET
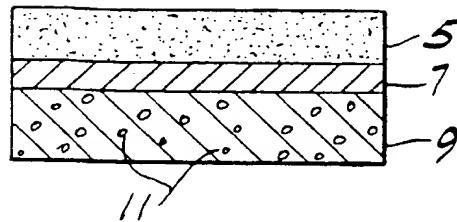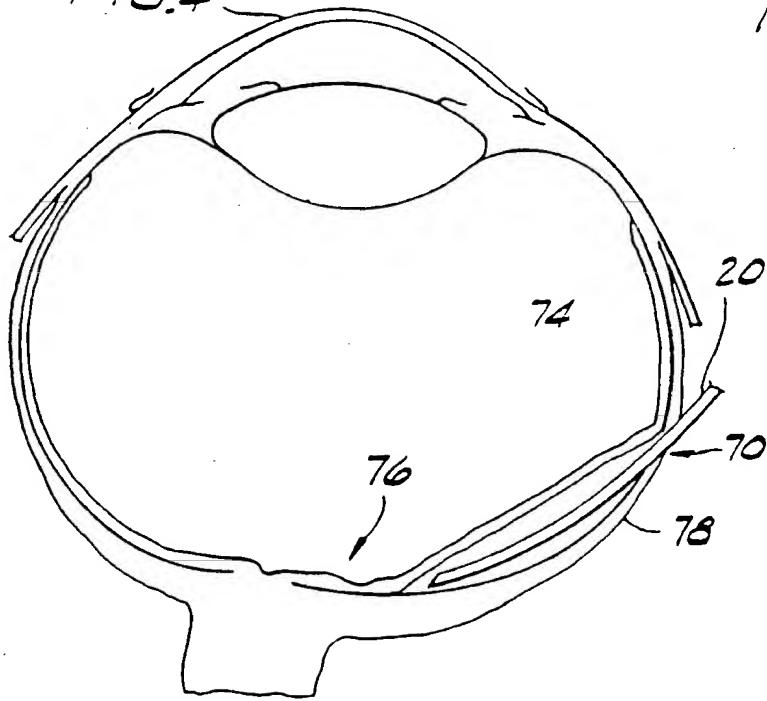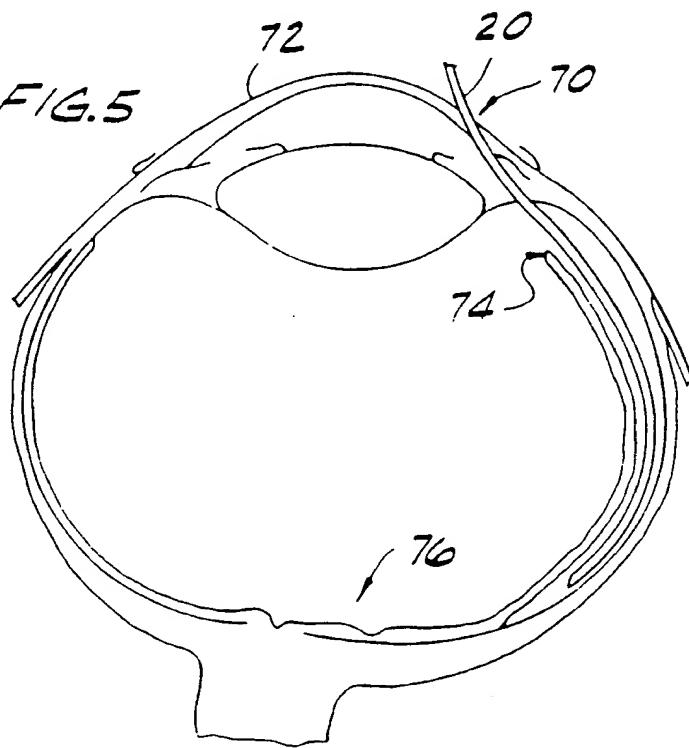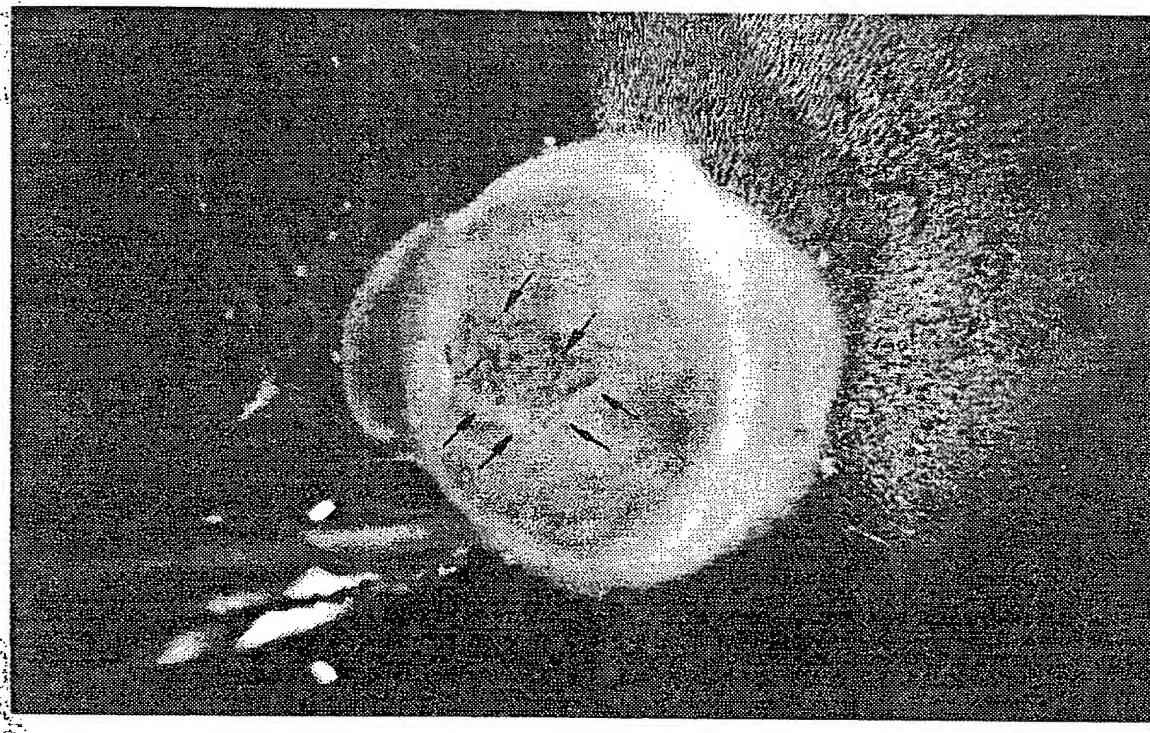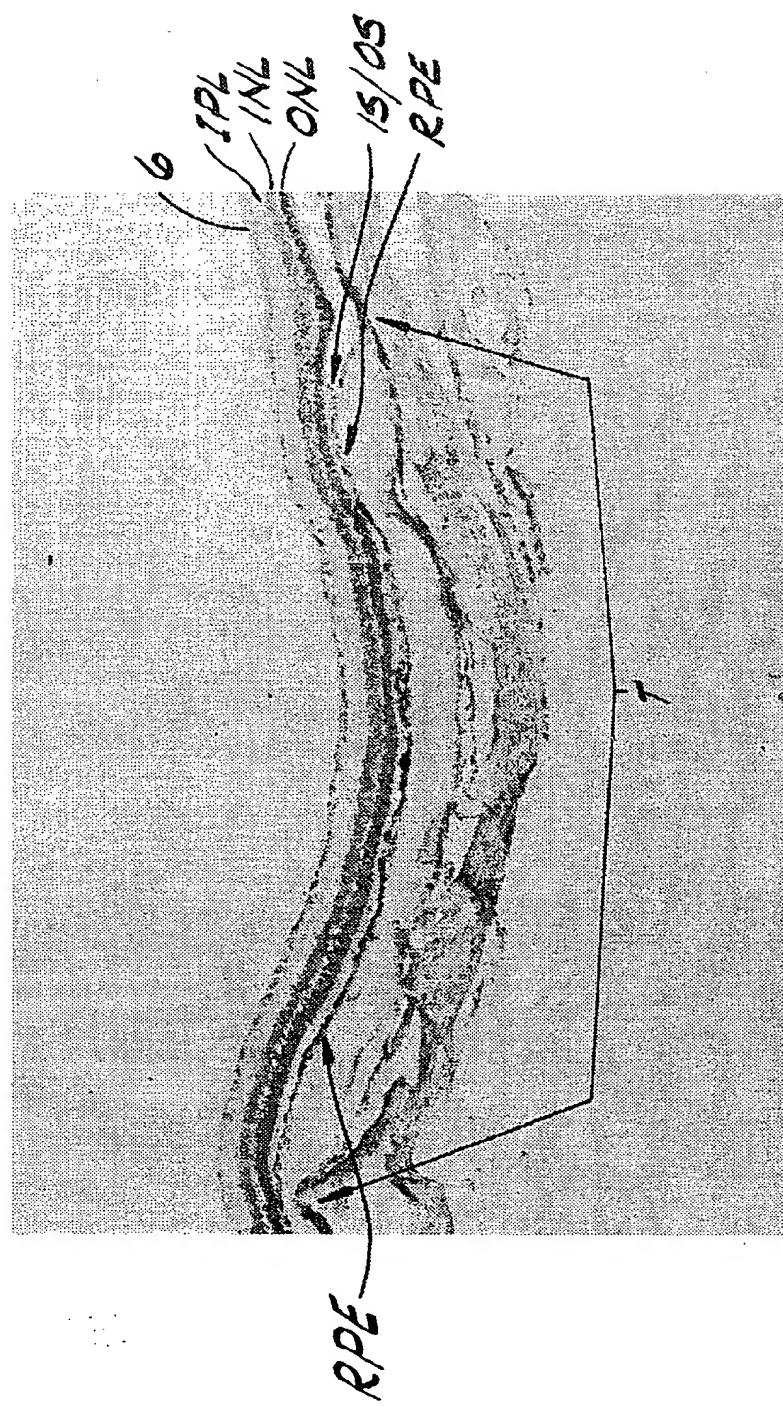
FIG.3

FIG.4

FIG.5

FIG.6

FIG.7

FIG.8

INL
OPL
ONL
IS
OS
A
RPE

FIG.9

G
ONL
RPE

RR   SF   RP   RD
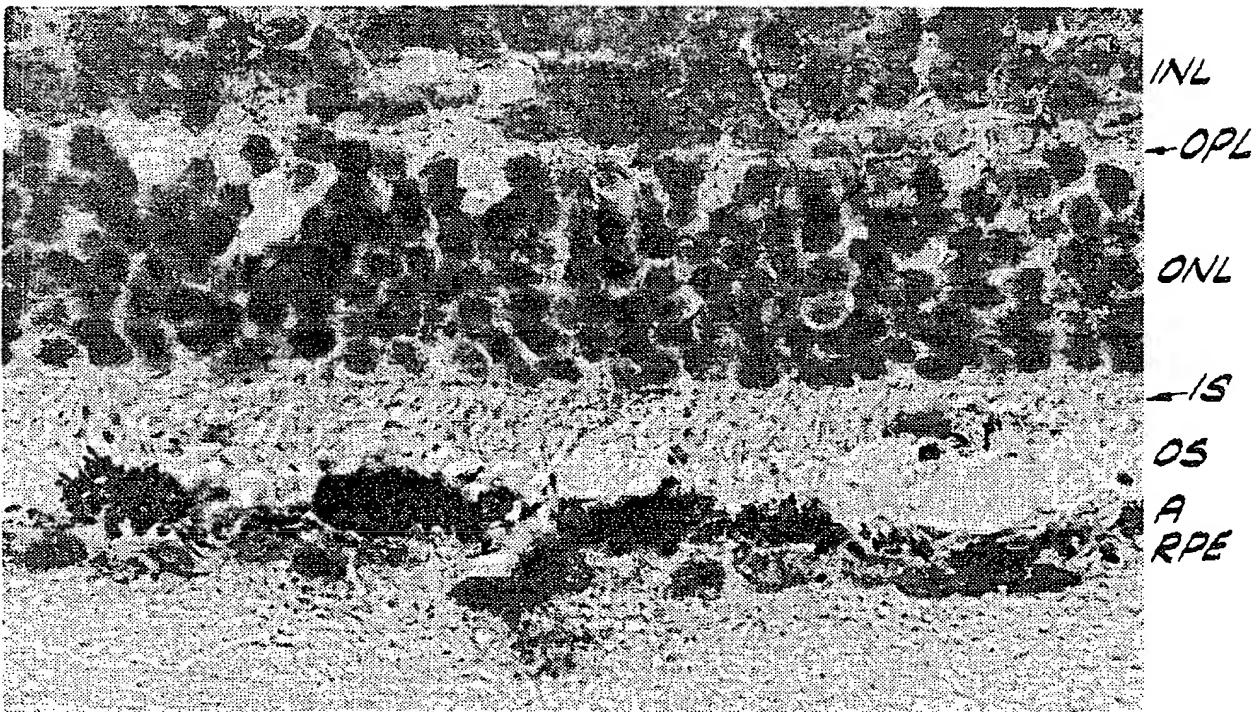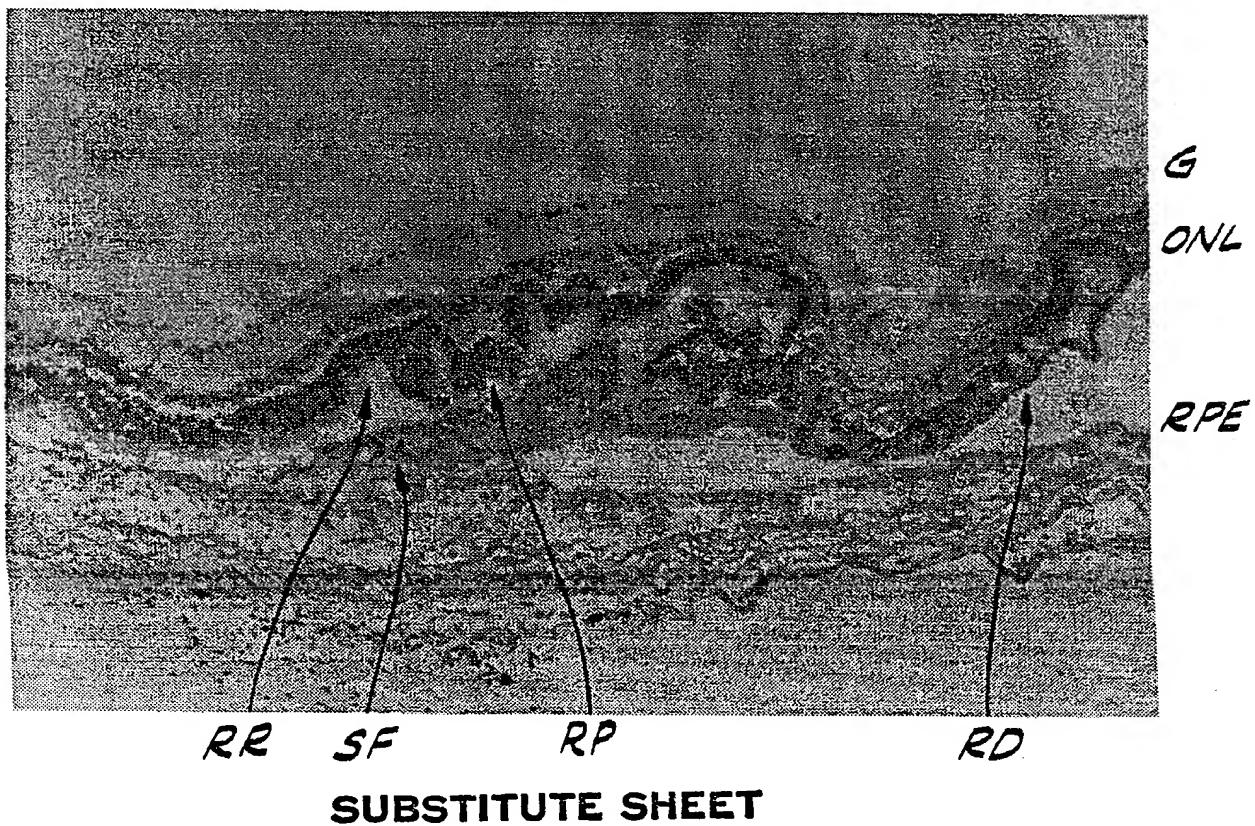
SUBSTITUTE SHEET

FIG.10
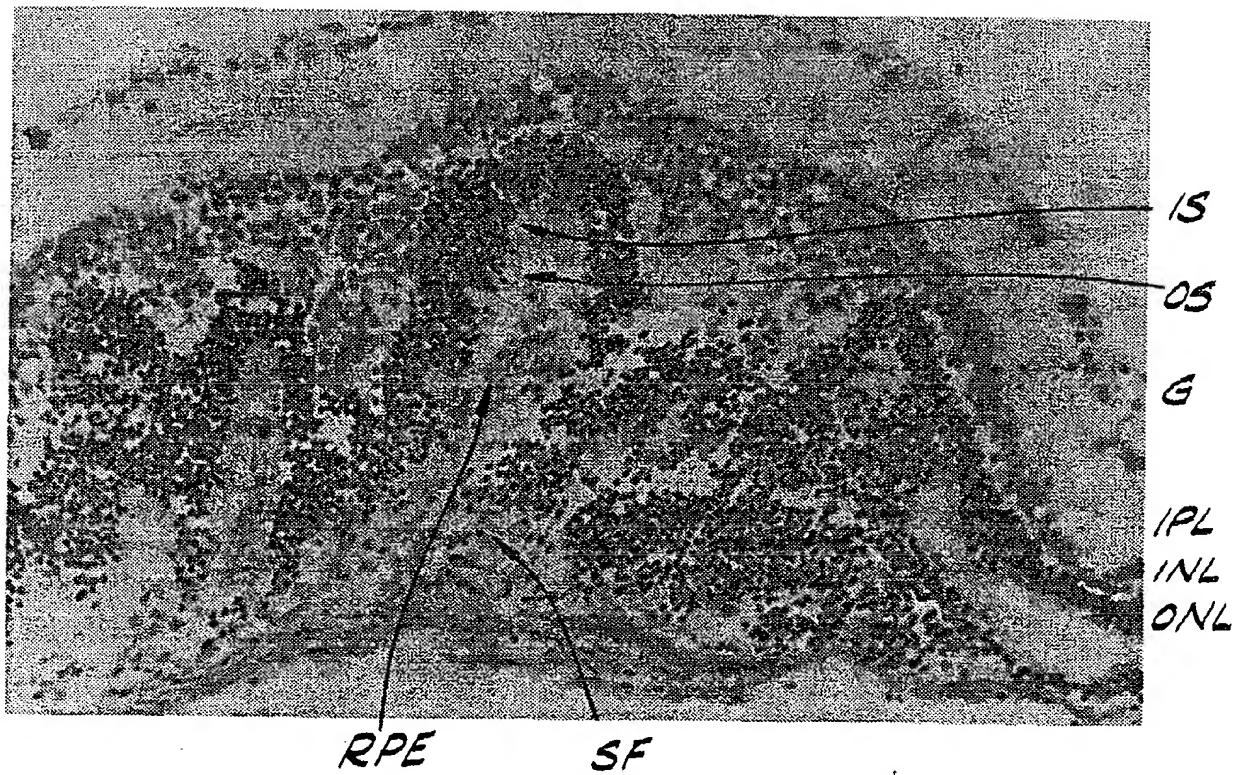
A. CLASSIFICATION OF SUBJECT MATTER

IPC(5)   :C12N 5/00. 11/00; A01N 1/02; A61M 35/00; A61F 2/14
US CL   :435/240.2, 240.21, 1, 174; 604/294; 623/4

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/240.2, 240.21, 1, 174; 604/294; 623/4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,000,963 (Hefton) 19 March 1991, see entire document.	1-38
Y	US, A, 4,495,288 (Jarvis, Jr. et al) 22 January 1985, see entire document.	1-38
Y	US, A, 4,963,489 (Naughton et al) 16 October 1990, see entire document.	1-38
Y	US, A, 4,927,676 (Williams et al) 22 May 1990, see entire document.	1-38
Y	Polyscience, issued January 1990, "Biodegradable Polymers", page 365, see entire document.	1-38

[X] Further documents are listed in the continuation of Box C.   [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure. use. exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 July 1993	**11 AUG 1993**

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington. D.C. 20231	SUSAN M. DADIO
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Graefe's Archives for Clinical and experimental Ophthalmology, Volume 224, issued 1986, Radtke et al, "Pharmacological therapy for proliferative vitreoretinopathy", page 122-125, see entire document.	1-38
Y	Investigative Ophthalmology & Visual Science, Volume 30, No. 8, issued August 1989, Silverman et al, "Transplantation of Photoreceptors to Light-Damaged Retina" pages 1684-1690, see entire document.	36-38
Y	Current Eye Research, Volume 9, No. 2, issued 1990, Silverman et al, "Photoreceptor rescue in the RCS rat without pigment epithelium transplantation", pages 183-191, see entire document.	36-38
A	American Journal of Ophthalmology, Volume 80, No. 3, Part II, issued September 1975, Mueller-Jensen et al, "Autotransplantation of retinal pigment epithelium in intravitreal diffusion chamber", pages 530-537, see entire document.	1-38
A	Exp. Eye Res., Volume 47, issued 1988, Li et al, "Transplantation of retinal pigment epithelial cells to immature and adult rat hosts; short- and long-term survival characteristics", pages 771-785, see entire document.	1-38
A	Investigative Ophthalomolgy & Visual Science, Volume 30, No. 3, issued March 1989, Lopez et al, "Transplanted retinal pigment epithelium modifies the retinal degeneration in the RCS rat", pages 586-589, see entire document	1-38
A	Exp. Eye Res., Volume 48, issued 1989, Sheedlo et al, "Functional and structural characteristics of photoreceptor cells rescued in RPE-cell grafted retinas of RCS dystrophic rats", pages, 841-854, see entire documnet.	1-38
A	Investigative Ophthamology & Visual Science, Volume 24, issued July 1983, Anderson et al, "Retinal detachment in the cat: the pigment epithelial-photoreceptor interface", pages 906-926, see entire document.	1-38
A	Investigative Ophthamology & Visual Science, Volume 26, issued November 1985, Mayerson et al, "An improved method for isolation and culture of rat pigment epithelial cells" pages 1599-1609, see entire document.	1-38

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